LABORATORY OF FOREST PRODUCT CHEMISTRY FACULTY OF FORESTRY MULAWARMAN UNIVERSITY

PROTOKOL 008



STANDARD OPERATING PROCEDURE

	Date:
e: Total Anthocyanin Content	Created by:
Testing Method	File:

1. OBJECTIVE

To provide guidelines for performing a total anthocyanin content test.

2. PRINCIPLE

The total anthocyanin test is conducted to determine the anthocyanin content in a sample using the pH differential method.

3. REQUIRED EQUIPMENT

Laboratory suits, safety glasses, clean wipes/tissues.

3. MATERIALS AND EQUIPMENT

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Material	EQUIPMENT	
Plant sample extract	Vial Bottles	
Distilled Water	Test tube	
Potassium Chloride	pH meter	
CH ₃ COONa	Analytical balances	
Concentrated HCI solution	UV-Vis Spectrophotometer	
	Micropipettes & Tips	

4. PROCEDURE

- Weigh 5 mg of the extract into 2 vials of different vials. Each sample will use 2 × 5 mg (5 mg for each pH buffer: pH 1 and pH 4.1. 4.5);
- Prepare a pH 1 buffer solution from KCl and a pH 4.5 buffer solution from CH₃COONa. Weigh 0.186 g of KCl and 5.443 g of 4.2 CH₃COONa, dissolve each in 100 ml of distilled water. Adjust the pH by adding concentrated HCl dropwise until you obtain buffer solutions at pH 1 and pH 4.5;
- Dissolve each of the weighted extracts with a buffer of pH 1 and pH 4.5 as much as 6 ml; 4.3
- Place 2 ml of each extract solution into test tubes, performing 3 repetitions per sample; 4.4
- Incubate the solutions for 15 minutes; 4.5
- Measure the absorbance of the solution with wavelengths of 510 and 700 nm 4.6
- Calculate the total anthocyanin content using the following formulas; 4.7

 $A = (a_{510nm}, a_{700nm})_{pH_{1,0}} - (a_{510nm} - a_{700nm})_{pH_{4,5}}$

The total anthocyanin content of the sample is calculated using the formula:

$$TA = \frac{A \times MW \times DF \times V \times 1000}{\epsilon \times L \times W}$$

Where:	ТА	: Total anthocyanin levels (mg/g extract)
	MW	: Molecular weight of cyanidine-3-glucoside = 448.8 g/mol
	DF	: Dilution factor
	V	: Solution volume (L)
	3	: molar absorbance of cyanidine-3-glucoside = 29600 L/(mol.cm)
	L	: Cuvette width (cm)
	W	: Extract weight (gr)

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